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Multicenter Trial on Mother-to-Infant Transmission of GBV-C Virus

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Evidence indicates that the GBV-C or hepatitis G virus can cause persistent infection in humans, but little is known on the importance of vertical transmission. To assess the risk of mother-to-infant transmission and the clinical outcome of infected babies, we investigated 175 anti-HCV positive mothers and followed-up their children for 3–33 months. GBV-C RNA was detected by RT-PCR and anti-E2 antibody was assayed by EIA. Thirty-four (19.4%) women were GBV-C RNA positive and transmission occurred to 21 (61.8%) babies; 20 (95.2%) acquired GBV-C alone, and one (4.8%) GBV-C and HCV. Maternal factors such as intravenous drug use, HIV coinfection, HCV-RNA positivity, and type of feeding were not correlated with GBV-C transmission. GBV-C RNA remained persistently positive in all infected babies but one baby who seroconverted to anti-E2. Seven (35%) babies with GBV-C alone developed marginally elevated ALT; the baby with HCV and GBV-C co-infection had the highest ALT peak value (664 IU/l). Seven of the 141 (5%) babies born to the GBV-C RNA negative mothers acquired HCV and six (85.7%) had abnormal ALT. The mean ALT peak value was significantly higher ($P < 0.05$) for babies with HCV than for those with GBV- C. None of the children with GBV-C or with HCV became icteric. GBV-C is frequently present in anti-HCV positive women. The infection is transmitted efficiently from mother to baby and rate of transmission is much higher than that for HCV. GBV-C can cause persistent infection in babies but usually without clear evidence of liver disease. ***J. Med. Virol. 54: 107–112, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: viral hepatitis; vertical/perinatal transmission; GBV-C/HGV; HCV

INTRODUCTION

Two novel, potentially hepatotropic flavi-like viruses were identified recently in patients with liver disease and tentatively named GBV-C and hepatitis G virus (HGV) [Simons et al., 1995; Linnen et al., 1996].

Sequence identity analysis of these two viruses clearly indicated that GBV-C and HGV are separate isolates of the same virus of different genotypes [Muerhoff et al., 1996a]. In addition, the phylogenetic analysis of the aligned viral polyprotein sequences showed that GBV-C/HGV is related to two Flavivirus-like agents (GBV-A and GVB-B) isolated from tamarins and to hepatitis C virus (HCV) [Leary et al., 1996].

GBV-C/HGV genomic RNA has been detected in pa-

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tients with both acute and chronic hepatitis and in patients with fulminant hepatitis of unknown origin, but until now its causality has not been proved [Simons et al., 1995; Yoshiba et al., 1995; Linnen et al., 1996; Fiordalisi et al., 1996; Dawson et al, 1996; Heringlake et al., 1996; Alter, 1996]. GBV-C/HGV can establish persistent infection and has a world-wide distribution, being detectable in approximately 2% of blood donors and in up to 20% of high risk groups, such as intravenous drug users (IVDUs) and multiply transfused patients [Linnen et al., 1996; Simons et al., 1996]. Different immunoassays for the detection of antibodies to the viral envelope glycoprotein E2 (anti-E2) have been developed recently to examine the antibody prevalence in various populations [Tacke et al., 1997; Dille et al., 1997].

Like HCV and hepatitis B virus (HBV), GBV-C/HGV is clearly transmitted parenterally, although few data are available about the epidemiological importance of vertical/perinatal route in the dissemination of this virus [Feucht et al., 1996; Viazov et al., 1997].

To assess the rate of mother-to-infant transmission and the clinical outcome of GBV-C/HGV infection, we have examined 175 mothers at delivery and followed their newborn children prospectively. All mothers included in this study were anti-HCV positive, and 18 women were also co-infected with HIV.

METHODS

Between January 1994 and March 1997 GBV-C RNA was sought in 175 pregnant women (mean age 31 years, range 21–43 years) who were anti-HCV positive when admitted for labor at several antenatal clinics in Lombardy (North Italy), following a protocol in use since 1990 [Zanetti et al., 1995]. Of these women, 143 (81.7%; 95% C.I. 75% to 87%) were positive for HCV-RNA and 18 (10.3%; 95% C.I. 6.4% to 16%) were also co-infected with HIV.

Demographic information and data on exposure to known risk factors were collected with a pre-coded questionnaire.

To search for mother-to-infant transmission of HCV and GBV-C, babies were followed for 3–33 months (mean 10.9 months). Peripheral blood sampling, laboratory evaluations and clinical evaluations were scheduled at birth (within 2 weeks of life), at about 3 month intervals during the first year of follow-up, and then every 6 months.

Forty-six (26.3%; 95% C.I. 20.1% to 33.6%) mothers, including 14 of those co-infected with HIV, had elective caesarean delivery because of obstetric indications. In addition, 101 (57.7%; 95% C.I. 50% to 65.1%) babies, including all those born to HIV positive mothers, were not breast-fed.

HCV antibody was detected by an enzyme-linked immunosorbent assay system (EIA, Abbott Laboratories, Chicago, IL) and repeatedly positive samples were confirmed further by RIBA (Chiron Corporation, Emeryville, CA). HCV-RNA was assayed by RT-PCR with nested primers derived from the 5′-non-coding region of the viral genome [Puoti et al., 1992]. HCV genotypes were determined by a line-probe assay (INNO-LiPA, Innogenetics, Belgium).

GBV-C RNA was detected with the Abbott LCx® system in samples collected from mothers at delivery and from their infants on at least two different occasions (mean 3.5, range 2–7), with aliquots stored at −80°C.

Viral nucleic acid was extracted from 25 μl plasma by using of the QIAamp® Viral RNA Kit (Qiagen Inc., Chatsworth, CA). The manufacturer's recommended procedure was followed except that carrier RNA was reduced to 6.7 μg/ml in AVL Buffer. Twenty microliters (5 μl plasma-equivalents) of the purified RNA was used for PCR testing. PCR amplification of the viral RNA was accomplished with the Abbott LCx® system. Briefly, rTth polymerase was used in a one step format to convert RNA to DNA and amplify the resulting DNA [Meyers and Gelfand, 1991; Young et al., 1993]. Primers were chosen from the highly conserved 5′-UTR of the GBV-C virus [Muerhoff et al., 1996b]. An internal 5′-UTR hybridization probe was also included in the PCR mix to confirm amplification of the correct sequence. This probe had a Tm at least 10°C lower than the primers so it did not interfere with or participate in the PCR reactions during amplification.

The cDNA step was 60°C for 30 min and was followed by 35 PCR cycles of 94°C/40 sec and 63°C/60 sec. Following amplification, the PCR products were denatured at 95°C for 5 min and rapidly cooled to 12°C to bind preferentially the probe to the PCR product. The probe-PCR product complex was detected by microparticle-enzyme immunoassay on the Abbott LCx® Probe System Analyzer.

Immunoassays for detection of human antibody elicited to the envelope GBV-C glycoprotein were carried out at Abbott Laboratories [Dille et al., 1997]. Briefly, a glycosylated form of the GBV-C E2 protein was purified and used as the antigenic target for detection of human anti-GBV-C antibody. An indirect immunoassay was developed which employed E2 on a solid phase to capture antibody from human serum or plasma, followed by addition of an enzyme conjugated anti-human antibody for color development. Positive or gray zone results were confirmed for each serum by a sandwich type immunoassay using the E2 protein.

Liver function tests were carried out by routine methods. Babies with abnormal ALT (>40 IU/l) were also examined for hepatitis B surface antigen (HBsAg) and for IgM antibody to hepatitis A virus (HAV), Cytomegalovirus (CMV), and Epstein-Barr virus (EBV; Abbott Labs). HIV was detected by EIA (Abbott Labs, Chicago, IL) and confirmed by Western Blot (Diagnostic Biotechnology, GeneLabs, Singapore).

For HIV-infected mothers, the clinical stage was assigned according to the CDC classification (Centers for Disease Control and Prevention, 1992).

All women gave informed consent before entering the study.

Statistical analysis was undertaken by Fisher's exact test for frequencies. The confidence intervals (C.I.) at 95% were calculated by Fleiss quadratic method; for all results, the upper and lower C.I. limits for 95% are

TABLE I. Influence of Maternal Factors on GBV-C Transmission

Maternal predictor		No. infected / No. children	
HIV	+	4/8 (50%)	$P = 0.7$
	−	17/26 (65.4%)	
HCV-RNA	+	18/28 (64.2%)	$P = 0.7$
	−	3/6 (50%)	
History of i.v. drug use	yes	10/17 (58.8%)	$P = 1$
	no	11/17 (64.7%)	
Type of feeding	breast feeding	4/7 (57.1%)	$P = 1$
	formula feeding	17/27 (63%)	
Mode of delivery	vaginal	15/21 (71.4%)	$P = 0.2$
	caesarean	6/13 (46.2%)	

given. Group means were compared by the Student *t*-test.

RESULTS

Prevalence of GBV-C and Clinical Features in Infected Mothers

Thirty-four of 175 (19.4%; C.I. 14.0–26.2%) mothers were positive for GBV-C RNA and two (5.9%; C.I. 1.0–21.1%) also had anti-E2 antibody. The frequency of GBV-C RNA was higher among women with HIV infection (8/18; 44.4%) than among those without HIV (26/157; 16.6%; $P < 0.05$). Within the GBV-C RNA positive group, 28 mothers (82.3%; C.I. 64.8–92.6%) were HCV-RNA positive. In addition, three mothers (8.8%; C.I. 2.3–24.8%) had abnormal ALT values (mean 80 IU/l, range 56–123 IU/l) at delivery, nine mothers (26.5%; C.I. 13.5–44.7%) had a history of chronic hepatitis and the remaining 22 (64.7%; C.I. 46.5–79.7%) had no signs or symptoms of acute or chronic liver disease. Eight mothers (23.5%; C.I. 11.4–41.6%) were also HIV infected (1 class I, 3 class II, and 4 class III).

As for risk factors, 17 (50%; C.I. 32.7–67.2%) were IVDUs, 2 (5.9%; C.I. 1.0–21.0%) had been given transfusions, 2 (5.9%; C.I. 1.0–21.0%) were sexual partners of IVDUs, one (2.9%; C.I. 0.1–17.0%) was a health care worker, and 12 (35.3%; C.I. 20.3–53.5%) had no apparent risk factors.

Mother-to-Infant Transmission of GBV-C and Clinical Features of Infected Babies

GBV-C RNA was detected in 21 of the 34 babies (61.8%; C.I. 43.6–77.3%) born to GBV-C infected mothers. Neither baby born to the two mothers with coexistent GBV-C RNA and anti-E2 was infected. Passive anti-E2 was present in both babies at birth but disappeared at 3 months. One baby (4.8%; C.I. 0.2–25.9) acquired both GBV-C and HCV infections and 20 babies (95.2%; C.I. 74.1–99.7) were infected with GBV-C alone. As shown in Table I, there was no significant difference in the rate of GBV-C transmission between babies born to mothers with HIV coinfection and those born to mothers without HIV (50% vs. 65.4%; $P = 0.7$) or between babies born to mothers with or without HCV-RNA (64.3% vs. 50%; $P = 0.7$).

The rate of GBV-C infection was similar for babies born to mothers who nursed them and for those who were not (57% vs. 63%; $P = 1$). A somewhat lower rate of GBV-C infection was seen in babies delivered by elective caesarian section (46.2%) than in those born vaginally (71.4%), but the difference was not statistically significant ($P = 0.2$). Maternal drug use also does not appear to be a predictor of mother-to-infant GBV-C transmission, since rates of infections were comparable in babies born to IVDU and non-IVDU mothers (58.8% vs. 64.7%; $P = 1$).

None of the GBV-C infected babies became icteric or developed HBsAg or IgM antibodies to HAV, CMV or EBV during the follow-up.

Among the 20 babies who acquired GBV-C alone, viral RNA first became detectable between the first 2 weeks of life (two cases) and 3 months of age and remained continuously positive during the observation period (mean 10.4 months, range 3–19 months) in all but one who seroconverted to anti-E2 antibody at 18 months of age.

Seven of the 20 (35%; C.I. 16.3–59.1%) children with GBV-C alone (Fig. 1) developed marginally elevated ALT levels (mean peak 69 IU/l, range 50–91 IU/l) in concomitance with or just after the first appearance of GBV-CRNA, while the remaining 13 (65%; C.I. 40.9–83.7%) children had persistently normal ALT.

In the baby who acquired both HCV and GBV-C infections, GBV-C RNA was first detected in the first 2 weeks of life, while HCV-RNA became positive at 3 months. ALT were normal up to 3 months of age, then peaked at 6 months of age (664 IU/l), and remained persistently elevated in the presence of both GBV-C and HCV.

Finally, all babies who escaped GBV-C and HCV infections (13/34; 38.2%; C.I. 22.7–56.4%) had persistently normal ALT levels.

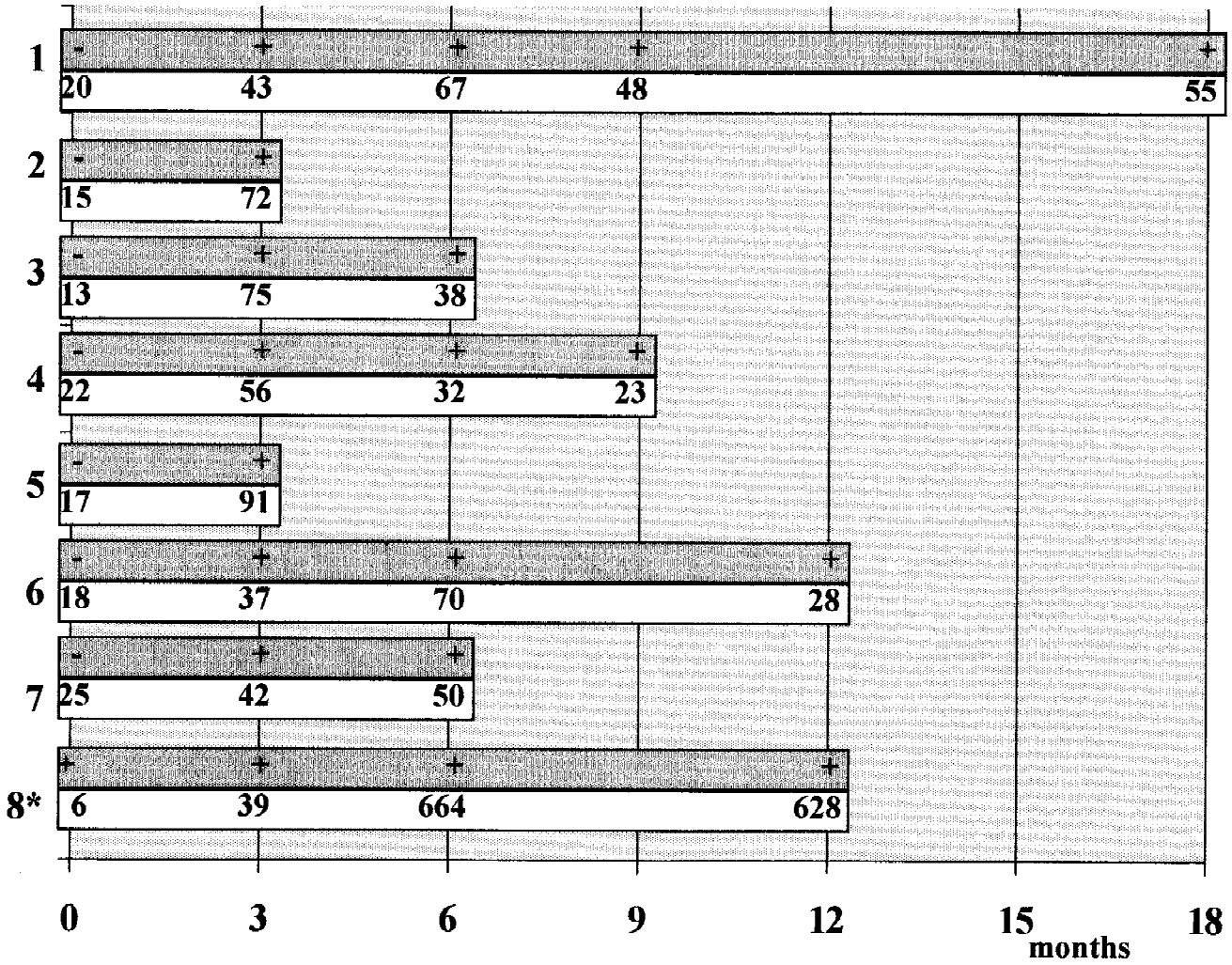

Fig. 1. Profiles of the GBV-C infected babies who developed abnormal ALT. Time 0 = at birth or within the first 2 weeks of life. Shaded bars = plus and minus signs indicate positive and negative GBV-C RNA. White bars = numbers indicate ALT (IU/l) values. *This baby acquired both GBV-C and HCV infectious.

HCV Infections in Babies Born to GBV-C Negative Mothers

Clinical features and risk factors of the 141 GBV-C negative mothers were comparable to those of the 34 mothers with GBV-C RNA and the follow-up rates of the two cohorts of babies were similar.

Seven babies (5%; C.I. 2.2–10.3%) acquired HCV infection, including one baby born to the subset of 10 mothers (10%; C.I. 0.5–45.9%) with HCV and HIV coinfection. Six of these infected children (85.7%; C.I. 42.0–99.2%) had abnormal ALT (mean peak 172 IU/l, range 66–303 IU/l; Fig. 2) and none became icteric or developed HBsAg or IgM to HAV, CMV and EBV during the follow-up. The HCV genotypes of infected babies (two cases had genotype 2a, two cases 3a, one case 1b, one case 4c/4d, and one case 4a) matched those of their mothers.

Finally, 94 of the 141 (66.7%; C.I. 58.2–74.2%) GBV-C RNA negative mothers were anti-E2 positive. All babies born to anti-E2 positive mothers had antibody at birth. The percentage with anti-E2 declined to 78% at 3 months and to 52% at 6 months, while at 12 months all babies tested (n = 33) had become anti-E2 negative.

DISCUSSION

Taken together, 128 of 175 (73.1%; C.I. 65.8–79.4%) mothers anti-HCV positive at delivery had signs of ongoing (viral RNA) or past (anti-E2 antibody) GBV-C infection. In all but two of the GBV-C exposed mothers, viremia and antibody were mutually exclusive. Thus, as for other flaviviruses such as dengue virus, Japanese encephalitis virus or yellow fever virus, GBV-C infected individuals are able to mount an immune response to the viral envelope glycoprotein (E2) which can clear the infection.

In our study, 34 mothers were GBV-C RNA positive and 64.7% (C.I. 46.5–79.7%) of them had no clinical or biochemical signs of liver disease. In the same way it was found that 63.1% (C.I. 54.5–71%) of the cohort of HCV-RNA positive mothers without GBV-C showed no signs of liver damage. Thus, it seems that during pregnancy GBV-C can coexist with HCV without causing more aggressive hepatitis.

The higher frequency of exposure to GBV-C infection in anti-HCV positive mothers than in the healthy female population (1.2% for GBV-C RNA and 15.4% for

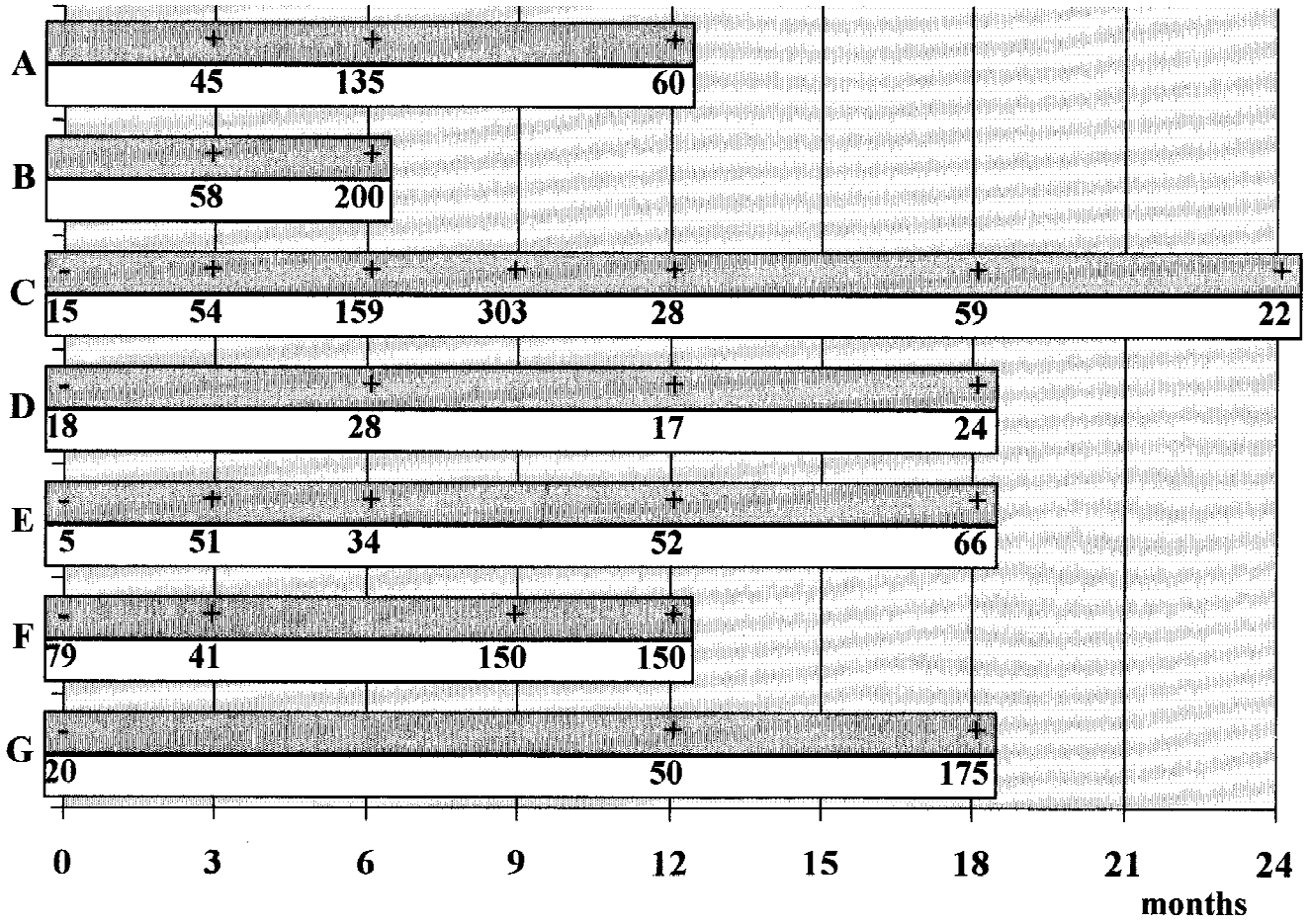

Fig. 2. Profiles of the babies who acquired HCV infection. Time 0 = at birth or within the first 2 weeks of life. Shaded bars = plus and minus signs indicate positive and negative HCV-RNA. White bars = numbers indicate ALT (IU/l) values.

anti-E2) of the same area and of comparable age (data not published), strongly suggests that GBV-C shares common sources of exposure with HCV. In agreement with this hypothesis, 65% of the GBV-C positive pregnant women showed known risk factors for parenterally transmitted viruses.

GBV-C is transmitted efficiently from the infected mother to baby and the rate of infection was found to be much higher than for HCV transmission (61.8% vs. 2.9% for the cohort of babies born to HCV and GBV-C dually infected mothers and vs. 5% for the cohort of babies born to HCV positive but GBV-C negative mothers, $P < 0.0001$).

The higher rate of mother-to-infant transmission of GBV-C compared with HCV has been also observed recently in a study carried out in Germany in which the difference was attributed to the higher levels of GBV-C RNA than HCV RNA found in the maternal sera [Viazov et al., 1997].

None of the 96 babies born to anti-E2 positive mothers (including the two babies of mothers with coexistent GBV-C RNA and anti-E2 antibody) became infected with GBV-C. All babies born to such mothers were anti-E2 positive at birth and lost antibody between 6 months and 1 year of age and were normal by clinical and laboratory examinations.

In this study, maternal predictors such as intravenous drug abuse, co-infection with HIV and type of feeding (maternal vs. bottle-feeding) were not found to correlate with GBV-C transmission. As for other bloodborne viruses, GBV-C transmission might be influenced by the mode of delivery [European Collaborative Study, 1994; Paccagnini et al., 1995; Lin et al., 1996]. A somewhat lower rate of neonatal infections was found in this study in babies born by caesarean section than for those born by vaginal delivery, but the difference was not statistically significant. Further studies are needed to clarify whether transmission of GBV-C is more likely to occur in utero, at the time of delivery or postnatally in order to adopt suitable preventive measures and to offer specific counselling.

Much effort has been spent on the study of the clinical significance of GBV-C infection but no final conclusive data are yet available [Miyakawa and Mayumi, 1997]. Several published studies have shown that GBV-C can develop into long-term infection and can be associated with cases of both acute and chronic hepatitis without any other cause of liver disease [Simons et al., 1995; Linnen et al., 1996; Fiordalisi et al., 1996]. However, there are still difficulties in determining whether GBV-C is the etiological agent of these diseases or is simply present by association only, since

most of the GBV-C viremic individuals have normal liver enzymes [Alter, 1996; Masuko et al., 1996; Alter et al., 1997; Alter et al., 1997].

Among the 20 babies who acquired GBV-C alone, viral RNA was positive persistently during the follow-up (10.4 months, range 3–19 months) in all but one, who seroconverted to anti-E2 at 18 months of age.

During the study, 13 (65%) of these babies had persistently normal ALT while seven (35%) had occasional slightly elevated ALT. In contrast, six of the seven (85.7%) babies who acquired HCV infection showed abnormal ALT. The mean ALT peak value was significantly higher among babies infected with HCV than among those with GBV-C (172 vs. 69 IU/l, $P < 0.05$).

Interestingly, the baby who had both GBV-C and HCV developed the highest peak level of ALT (664 IU/l). Whether in this baby each virus has contributed to worsening the outcome of the infection caused by the other agent remains open.

In conclusion, our study shows that GBV-C infection is highly prevalent in anti-HCV-positive pregnant women and that the frequency of mother-to-infant transmission of GBV-C is much higher than that of HCV. Whether such high rate of neonatal GBV-C infection has been influenced by the maternal coexistence of HCV infection or it occurs also from mothers without HCV remains to be determined.

GBV-C infection is generally silent and chronically infected babies usually have normal or only occasional slightly raised liver enzyme levels, without clear evidence of liver damage. Evidence also suggests that GBV-C can clear over time with seroconvertion to anti-E2 antibody. Whether this antibody can confer long-lasting protection against re-infection is not yet known [Simons et al., 1996].

Long-term follow-up of GBV-C infected babies is required to establish the clinical evolution of these infections.

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